Technical Manual

Human Influenza B Virus (BIV) IgM ELISA Kit

- Catalogue Code: HDES0051
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Capture-ELISA as the method to detect the BIV-IgM in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with Mouse-anti-human IgM (μ chain). Samples are added to the ELISA Microtiter plate wells and the IgM antibody in which will be captured. Free components are washed away (including the specific IgG antibody). Then add the HRP conjugated BIV antigen to each well and incubate. The HRP conjugated BIV antigen will react with the "anti-μ chain –BIV IgM" and form the "anti-μ chain –BIV IgM-HRP conjugated BIV antigen" compound. The TMB substrate is added after washing to initiate the color developing reaction. The presence of BIV IgM antibody can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

2. Kit components

Item	Specification
ELISA Microtiter plate	96 wells
Positive Control	1 mL
Negative Control	1 mL
HRP Conjugated Working Solution	12 mL
Sample Diluent	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Experimental instrument

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized water
- Absorbent paper

4. Requirements of sample

1. **Serum:** Human serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated. Avoid of samples with hyperlipidemia (triglyceride≥20 g/L), hemolysis (hemoglobin ≥10 g/L) or jaundice (bilirubin ≥0.2 g/L). Obviously contaminated samples can't be detected.
2. Do not use heated inactivated samples. Heat inactivation will degrade antibodies.
3. Samples can be stored at 2~8° C for one week. If samples not tested in a week, store them at -20° C and avoid freeze-thaw cycles.
4. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

5. Assay procedure

Restore all reagents and samples to room temperature (25° C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8° C.

1. **Add sample:**
   (1) Take out micro-plate and mark it, reserve 1 well for blank control (empty), 3 wells for negative control, 2 well for positive control (100 µL control serum for each well). (Blank well is not necessary for dual-wavelength detection)
   (2) Dilute the tested **Serum** with **Sample Diluent** at 1:10 into sample well (add 100 µL of sample diluent and add 10 µL of serum sample), mix fully.
   (3) Gently tap the plate to ensure thorough mixing.
2. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
3. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with wash buffer and immerse for 30-60 sec each time.
4. **HRP conjugate:** Add 100 µL of HRP Conjugate Working Solution to each well except the blank control well.
5. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
6. **Wash:** After incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with wash buffer and immerse for 30-60 sec each time.
7. **Add substrate:** Add 50 µL of Substrate Reagent A and 50 µL of Substrate Reagent B to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 15 min at 37° C in dark.
8. **Stop reaction:** Add 50 µL of Stop Solution to each well, gently tap the plate to ensure

thorough mixing.

9. **OD Measurement:** Set the Micro-plate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not needed when using dual wavelength 450 nm/630 nm for detection.

6. Reference value

1. **Result analysis**
   (1) Use each test result independently. Determine the result according to the Cut Off value.
   (2) Calculate the Cut Off: Cut Off(C.0) = 0.10 + negative control(NC) average A value (when NC average $A_{450} < 0.05$, calculate at 0.05; while NC average $A_{450} \geqslant 0.05$, calculate at the actual value).
2. **Quality control**
   (1) Blank well (just chromogenic agent and stop solution) absorbance $\leqslant 0.08$.
   (2) Positive control (PC) $A_{450} > 0.80$.
   (3) Negative control (NC) $A_{450} < 0.10$.
   The experimental result is valid if quality control is valid.
3. **Determination of results**
   (1) Positive result: Sample absorbance $\geqslant$ Cut Off.
   (2) Negative result: Sample absorbance $<$ Cut Off.

7. Interpretation of results

1. Negative result indicates there is no BIV-IgM antibody detected in samples, while positive result means the opposite.
2. The positive result of BIV-IgM antibody is an important index of BIV infection.

8. Limitations of test method

1. All high sensitivity immune experiment system exists potential non-specificity. Therefore, unacceptable positive results may be caused by biological false positive of ELISA method.
2. Any positive result should be determined combined with clinical information.

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